BIOLOGICAL CONTROL OF *CANNABIS SATIVA:* A SUMMARY OF THE PROJECT

2/6/76
Arthur H. McCain

Purpose and Objectives

The purpose of this study was to determine the feasibility of using Fusarium wilt, caused by *Fusarium oxysporum* f. *cannabis* Noviello and Snyder, to control unwanted *Cannabis sativa* L. The objectives of the research were to determine: (i) the genetic stability and host specificity of the *F. oxysporum* f. *cannabis;* (ii) methods of producing and applying inoculum; (iii) effectiveness of the fungus in the field, (iv) survival of the fungus in soil and reinfection of host plants in succeeding seasons; and (v) special precautions or techniques for controlled use of the fungus as a pathogen directed to disease production in a specific host.

Previous Work

Fusarium wilt of hemp (*Cannabis sativa* L.) causes serious losses in the cultivated crop in Italy (1). The disease was observed in a field at Alvignano, Caserta, in 1959 and in some other localities in 1960. The foliage of infected plants yellows, wilts, dries up and hangs on the plants. A dark-brown discoloration of the vascular system attends the foliage symptoms. As a rule, infected plants are killed. In the field, the disease is first noted in June when the plants are three months old, and by the end of June the disease is very evident. The disease is known to occur naturally only in Italy.

The disease is caused by *Fusarium oxysporum* f. *cannabis* Noviello and Snyder. The form of the fungus that attacks *C. sativa* is highly specialized and is not known to infect other crop plants. *Fusarium oxysporum* forms are restricted in pathogenicity to a very limited host range (2).

For example, the tomato form only attacks tomato. In California and elsewhere other crop plants, such as watermelons, alfalfa, beans (which are susceptible to different specialized forms of the fungus) grown in fields known to harbor the tomato form are not affected. Hops (*Humulus lupulus* L.), one of the closest crop plants related to *Cannabis*, are not known to be susceptible to a Fusarium wilt disease anywhere in the world.

References:

1. Noviello, Carmine and William C. Snyder. 1962. Fusarium wilt of hemp. Phytopathology 52: 1315-1317.
2. Snyder, W. C. and H. N. Hansen. 1940. The species concept in *Fusarium*. Amer. J. Bot. 27: 64-67.
3. Nash, Shirley M. and William C. Snyder. 1962. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. Phytopathology 52: 567-572.

Genetic Stability

The area devoted to the cultivation of hemp in Italy has declined from a high of 20,000 hectares to the present level of approximately 80 hectares in all of Italy. The crop is no longer grown in the Caserta area where Fusarium wilt was severe in 1959. Most of the hemp in Italy is now grown in an area near Naples. The susceptible varieties grown in 1959 and 1960 are no longer cultivated. The variety grown in 1972 through 1975 is 'Super Fibra' (formerly 'T-4').

Hemp fields in Italy were visited in 1972, 1973, 1974 and 1975. Hemp plants exhibiting Fusarium wilt were difficult to find but some infected plants were found and isolations were made from stem tissues. The fungus was present in most of the selected, infected plants. In 1972 the fungus

was recovered from plants from three different fields. The incidence of the disease was very low; possibly less than 0.1%. *Sclerotium rolfsii*, broomrape, and stem-boring insects made it difficult to identify plants with Fusarium wilt. We now know that 'Super Fibra' is very resistant to Fusarium wilt and this explains the low levels of the disease encountered. In retrospect it is surprising that it was even possible to find the disease.

The isolates of the fungus recovered from the hemp plants were similar in appearance on agar medium. Pathogenicity tests were conducted using four Italian hemp varieties available: 'Super Fibra', 'Carmagnola Orifinaria', 'Eletts Campana' and 'Carmagnola Selezionata'. Roots of seedlings were repotted in a sand-peat potting mixture and maintained in a growth chamber at 80°F. Very few plants exhibited Fusarium wilt symptoms; however, the fungus was recovered from two plants of 'Carmagnola Selezionata'. Twenty single-spore cultures were established from the re-isolated fungus. The single-spore isolates were all similar in appearance and did not differ from other isolates of the fungus. The characteristics of the fungus in culture were very stable. The fungus appears the same on agar medium today as it did when first isolated in 1972.

Pathogenicity tests using the four Italian hemp varieties, Iowa variety, and Iran variety did not reveal differences in pathogenicity among the fungus isolates; thus there appears to be a single race or pathotype of the fungus which is genetically stable.

Varietal Susceptibility

Twenty-two varieties or seed collections from different parts of the world were evaluated for disease susceptibility. We had determined that

wounds were not necessary for infection and that plants became infected when seeds were sown in infested soil. The inoculum level was very high, in excess of 10,000 fungus propagules/gram of soil. The tests were conducted in a growth chamber in continuous light and 8 hours at 21°C and 16 hours at 27°C. Seedlings emerged in 4 to 6 days depending upon the age and vigor of the seed. The first dead seedlings appeared 10 to 12 days after sowing of the seed. Varieties or seed collections from Mexico (three different collections), Pakistan, Turkey, Thailand, India, Nepal, South Africa, Czechoslovakia, Poland and Iran were all highly susceptible to the disease and in general there were no survivors or escapes in the growth chamber study. The four Italian hemp varieties were resistant and only 10 to 20% of the seedlings became infected. The survivors generally developed normally and were uninfected at the termination of the experiment. The fungus was consistently isolated from dead or diseased plants of the susceptible varieties. Seeds obtained from Portugal were also resistant. Mr. Mellisurgo of the Italian Hemp Growers Association believes that the Portugal seed is of Italian origin, that is grown in Italy, and is most likely 'Super Fibra'.

Seeds from escaped hemp plants collected in Iowa were intermediate in susceptibility. When 'Iowa', 'Iran' and 'Super Fibra' were compared, 100% of the 'Iran' plants were dead after 30 days, 70% of the 'Iowa' plants were dead, and 20% of the 'Super Fibra' plants were dead at the conclusion of the experiment. Fifty percent of the 'Iran' seedlings were dead in 14 days, while 19 days passed before 50% mortality occurred in the 'Iowa' seedlings. The 'Super Fibra' seedlings that died did so mostly (90%) in the first 20 days.

Host Specificity

A variety of plants were tested for susceptibility to the fungus. Seeds were either planted in heavily infested soil (excess of 20,000 propagules/gram) or were transplanted into infested soil. Isolations were made from any plant that differed from the non-inoculated control plants. The fungus was never isolated from any plants other than *C. sativa* 'Iran' control plants grown at the same time in infested soil. The 'Iran' plants always became infested. The plants were maintained in a greenhouse at 27°C. Tomatoes, cotton, and carnations were also grown in soil infested with their respective *F. oxysporum* formae speciales and became infected.

The following plants were evaluated.

Scientific Name	Common Name	Variety
Allium cepa	onion	Tulelake 401
Allium sativum	garlic	"Safeway"
Anethum graveolens	dill	—
Apium graveolens var. *dulce*	celery	Fordhook
Arachis hypogaea	peanut	Jumbo Virginia
Asparagus officianalis	asparagus	UC72
Avena sativa	oats	Montezuma
Beta vulgaris	beet	Ruby Queen
Beta vulgaris	sugar beet	F56
Beta vulgaris var. *cicla*	chard	Ocelga
Brassica juncea	mustard	Foodhook Fancy
Brassica oleracea var. *botrytis*	cauliflower	Early Snowball
Brassica oleracea var. *capitata*	cabbage	Wisc. Golden Acre
Brassica rapa	turnip	Purple Top
Callistephus chinensis	aster	Burpeana
Capsicum frutescens	pepper	Burpee Tasty Hybrid
Carthamus tinctorius	safflower	Gila
Chrysanthemum morifolium	chrysanthemum	Iceberg
Cicer arientinum	chickpea	—
Citrilus vulgaris	watermelon	Klondike
Cucumis melo	muskmelon	Burpee 199
Cucumis sativus	cucumber	Ashley
Cucurbita pepo	zucchini	Italian Marrow
Daucus carota	carrot	Danvers Half Long

Scientific Name	Common Name	Variety
Dianthus caryophyllus	carnation	Red Scania
Eschscholzia californica	California poppy	—
Gossypium hirsutum	cotton	SJ-1
Glycine max	soybean	Kanrich
Helianthus annuus	sunflower	Mammoth
Hibiscus esculentus	okra	Clemson Spineless
Hordeum vulgare	barley	Calif. Mariout
Humulus lupulus	hop	California
Ipomea batatas	sweet potato	"Safeway"
Lactuca sativa	lettuce	Great Lakes
Linum usitatissimum	flax	New River
Lycopersicon esculentum	tomato	Bonny Best
Matthiola incana var. *annua*	stock	—
Medicago sativa	alfalfa	Moopa
Morus alba	white mulberry	—
Morus nigra	black mulberry	—
Oryza sativa	rice	Colora
Papaver rhoeas	corn-poppy	—
Papaver somniferum	opium poppy	Iran
Phaseolus aureus	mungbean	Yuba
Phaseolus vulgaris	bean	Calif. Red Kidney
Pisum sativum	pea	Thomas Laxton
Raphanus sativus	radish	Scarlet Globe
Solanum melongena	eggplant	Early Beauty
Solanum tuberosum	potato	Russet Burbank
Sorghum vulgare	milo	Double Dwarf
Spinacia oleracea	spinach	America
Trifolium repens	white clover	—
Triticum vulgare	wheat	Pitic 62
Vicia sp.	vetch	Lana
Vicia fava	fava bean	—
Vigna sinensis	cowpea	California 3
Zea mays	sweet corn	Golden Bantam
Zinnia elegans	zinnia	Bodger

No crop plants, including tobacco, tomato, bean, wheat, grape, alfalfa, corn, potato, and squash, grown in rotations with hemp in Italy have become infected by the fungus.

Inoculum Production

Various animal feeds (including alfalfa meal, almond hulls, rolled barley, sugar beet pulp, cottonseed meal, rolled milo, safflower meal, soybean oil meal, malted barley, and barley straw) were evaluated as culture

media for production of inoculum. Alfalfa meal, soybean meal and cotton seed meal were superior to the other feeds in the numbers of chlamydospores and conidia produced. The superiority of these materials may be related to the high protein content. Since these materials tended to cake and the fungus did not grow well into the autoclaved medium, they were diluted with barley straw to facilitate aeration and separation.

The fungus was grown on medium composed of barley straw *(Hordeum vulgare)* and soybean meal *(Glycine max)* from which the oil had been extracted. The barley straw was milled so that the largest pieces were 30 mm but most pieces were smaller. One hundred and sixty grams of soybean meal were mixed with 800 g of barley straw and 2 liters of water. The mixture was thoroughly stirred and placed in large glass flasks plugged with cotton for sterilization. It was found necessary to autoclave the medium twice in order to eliminate heat-resistant bacteria. The first autoclaving was at 121°C for one hour followed in 24 hours by the second autoclaving for 1 hour at 132°C.

After cooling the medium was inoculated using conidial suspensions obtained from cultures of the fungus grown on potato-dextrose agar. After 2 to 4 weeks at 20-22°C the inoculum was removed from the flasks and dried for 5 to 7 days in a laboratory room at 20-22°C, relative humidity 40-50%. After drying the inoculum was broken up by hand, placed in polyethylene plastic bags and stored in the laboratory or in a freezer at -10°C. No attempt was made to maintain the inoculum in a sterile condition after removal from the culture flasks.

The inoculum was composed of particles of the straw and soybean meal colonized by the fungus — mycelium, conidia, and chlamydospores.

The inoculum used in the 1974 field trials in Portici was 3 to 4

months old. No appreciable loss of viability has been detected in inoculum kept at 20-22°C for one year or at -10°C, although one might expect a slow loss of viability at 20-22°C.

Application of Inoculum

In greenhouse and growth chamber studies the prepared inoculum was incorporated into soil at various levels. The soil was moistened and left undisturbed for two weeks. The number of propagules of the fungus per gram of soil was determined by soil dilution plating. Three hundred propagules per gram of soil were present when 0.025 g of inoculum was mixed with 1000 g of air-dry sandy loam soil; 2900 propagules/g at 0.050 g/1000 g soil; 11,300 propagules/g at 0.250 g/1000 g soil; 18,500 propagules/g at 0.500 g/1000 g soil; 67,000 propagules at 2.50 g/1000 g soil; and 95,000 propagules/g at 5.00 g/1000 g soil.

When susceptible 'Iran' was sown in soil containing different levels of the fungus, the time required for total kill of the seedlings was proportional to the inoculum level. At 7000 propagules/g of soil, 100% mortality was reached in 9 days; at 1400 propagules/g, 15 days elapsed before 100% mortality was reached; 22 days at 700 propagules/g; and 47 days at 70 propagules/g. When the propagule count was 7 propagules/g, 25% mortality occurred after 47 days when the experiment was terminated.

Effectiveness of fungus in the field. In April 1974, four fields in Italy were inoculated using three levels of air-dry straw-soybean inoculum. The inoculum was distributed by hand and mixed into the top few inches of soil with a hoe to prevent loss by blowing and so that the hemp plants would be certain to make contact with the inoculum.

Ten grams per square meter were distributed in the plot at Vitulazio.

The soil at Vitulazio is a loamy clay. The inoculated plot was seeded to 'Carmagnola Selezionata' ('CS') and 'Super Fibra' ('SF'). No *Fusarium*-infected plants were observed at harvest in July in this field.

Three levels of inoculum were applied in the Marcianise field which was a sandy loam soil. The hemp varieties 'CS' and 'SF' were planted in April. Very few plants exhibited Fusarium wilt symptoms and there was no correlation with the quantity of inoculum applied.

The Alvignano field was inoculated with 10 g/m^2 and seeded with 'SF' and seeds from 'Iran'. There was insufficient seed of 'Iran' to plant the Marcianise and Vitulazio fields in 1974. When final disease counts were made in August, 71% of the 'Iran' plants had died while only two plants of 'SF' in the inoculated plots were found infected. The surviving 'Iran' plants in the inoculated plots were 1.45 m in height while those in the noninoculated control plots were 3.60 m in height. The surviving plants were probably infected but isolations were not made from these plants. Isolations confirmed that the dead or dying plants were infected by the fungus. By June 14, 29% of the 'Iran' plants had died. It was clear that the inoculum was effective and that 'SF' was highly resistant to the disease.

There were two inoculum experiments conducted at Portici in 1974. One was in a field of fine sandy loam and the other in large ceramic pots containing the same soil. Three levels of inoculum were applied: 1, 10 and 30 g/m^2. Three varieties of hemp 'CS', 'SF' and 'Iran' were seeded on 20 April 1974. There were four replications, and diseased (dead) counts were made periodically and the infected (dead) plants removed to facilitate counting. Removal of the dead plants of course reduced the potential inoculum for succeeding crops. The first infected plants were removed

from the plots on June 4. Final counts were made on August 3. The percent dead 'Iran' plants for the three levels of inoculum (1, 10 and 30 g/m^2) were 50, 94.2 and 93.7, respectively. No plants in the noninoculated plots became infected. The level of disease in the Italian hemp varieties 'CS' and 'SF' were very low: only 2% of the 'CS' variety died in the plots receiving the highest inoculum level of 30 g/m^2 and only 1% of the 'SF' variety died at this inoculum level.

The 'Iran' plants that did not die in the Portici field were stunted. The plants in the noninoculated plots averaged 1.3 m in height while those in the 1 g/m^2 plots were 0.96 m; 0.71 m in the 10 g/m^2 plots. When height measurements were made, there were insufficient plants to measure in the 30 g/m^2 plots. The results from the experiment in the ceramic pots was almost identical to the field trial.

Downy mildew (*Pseudoperonospora cannabina*) was severe on the 'Iran' variety and copper fungicides were applied to control the disease. Downy mildew was not severe on the Italian hemp varieties and did not require sprays which were, however, applied.

Survival of the fungus in soil. An experiment was conducted in a sandy loam field at the University of California San Joaquin Valley Agricultural Research and Extension Center at Parlier. Two levels of straw-soybean inoculum, 1 g/m^2 and 10 g/m^2, were incorporated into the top 7.5 cm of soil on April 16, 1974. A crop of corn was planted which was harvested in October. The inoculated plots were sampled periodically and the number of propagules of *F. oxysporum* f. *cannabis* determined. *Fusarium oxysporum* f. *cannabis* was readily distinguishable from the native *F. oxysporum* present.

The average number of propagules in the inoculated plots declined

from the initial levels of 1500 g/in the 1 g/m^2 plots and 90,000 in the 10 g/m^2 plots to a just detectable level (19/g) in the 1 g/m^2 plots and 250/g in the 10 g/m^2 plots 200 days after incorporation. Monitoring of the fungus level was discontinued in the 1 g/m^2 plots after 200 days. After 460 days the propagule level had declined to 40/g in the plots that had received 10 g/m^2 of the straw-soybean inoculum.

Barley was planted in the spring of 1975. *Fusarium oxysporum* f. *cannabis* in the inoculated plots increased in numbers in senescent barley roots along with other *Fusarium oxysporums*, and then declined again as the soil became dry. The last propagule count was made in August 1975, 16 months after inoculation. The fungus was still detectable at 125 propagules/g. Survival of the artificial, straw-soybean inoculum in the absence of host plants was adequate to ensure the presence of the fungus in the soil for at least one growing season.

The experimental work area was fumigated with methyl bromide gas beneath a polyethylene cover in November 1975. The fungus is most likely no longer present in this field.

Reinfection of host plants. Three of the same fields in Italy inoculated and planted with hemp in 1974 were replanted in 1975. The susceptible 'Iran' variety was planted in each field in addition to seeds which were purchased in Portugal.

In the Vitulazio field where no disease was detected in the resistant Italian hemp varieties in 1974 there was 6.2% Fusarium wilt in the 'Iran' in late July 1974 and little or no disease in 'Portugal' variety. The fungus was able to survive or maintain itself on the resistant varieties.

In the Marcianise field where in 1974 there were scattered infections in the resistant Italian varieties without correlation to inoculum level;

there was severe disease in 'Iran' and the amount of disease (mortality) was correlated with the 1974 inoculum levels. The percent mortality in July 1975 in the 1 g/m^2, 10 g/m^2 and 30 g/m^2 rates of inoculum (applied in April 1974) were 36%, 87% and 93%, respectively. There was 22% mortality in the noninoculated plots, suggesting some natural spread of the fungus. The infection in the noninoculated control plots was heaviest near the inoculated areas.

The Portici plots were replanted with 'Iran' hemp. Disease counts were made periodically. The disease appeared first in the inoculated subplots where the 'Iran' variety had been in 1974. The amount of disease in 1975 was correlated with the inoculum levels in 1974. On July 11, 1975, the average amount of disease (mortality) in the 1 g/m^2, 10 g/m^2, 30 g/m^2 and noninoculated plots was 74%, 90%, 92%, and 48%, respectively.

The ceramic containers were also replanted using both 'Iran' and 'Portugal' seeds. There was no disease in the 'Portugal' variety and 90-95% in 'Iran'. There was no infection in 'Iran' in the noninoculated pots since there was no contamination of these pots from soil movement.

Seed-borne inoculum. Susceptible 'Iran' plants that became infected in field plots in Italy did not produce seed. However, in most Fusarium wilt diseases viable seeds do not become infected but the fungus contaminates the seed as bits of infected tissue mixed with the seed or as spores that cling to the seed.

To demonstrate that *F. oxysporum* f. *cannabis* could also be seed-borne, moistened 'Iran' seeds were mixed with straw-soybean inoculum and large visible pieces of inoculum removed. The inoculated seed was indistinguishable from the noninoculated seed. After air-drying the seeds were planted in pasteurized soil. Fifty percent of the inoculated seeds became

infected and died within 30 days when the experiment was terminated.

Safety of Inoculum

Inoculum composed of straw-soybean meal colonized by the fungus was tested for mycotoxins and mutagens. Chloroform extracts of the inoculum were injected into mice with no adverse effects. The chloroform extracts were also used in culture medium of bacteria strains sensitive to mutagens. These tests were conducted by Dr. Leonard Bjeldanes, Nutritional Sciences, University of California, Berkeley. (A more detailed report is being prepared.)

Summary

Fusarium wilt caused by *Fusarium oxysporum* f. *cannabis* is a suitable pathogen for use in the biological control of unwanted *Cannabis sativa*. Inoculum appropriate for large scale field use is grown on a mixture of 80% barley straw and 20% soybean or alfalfa meal. One gram per square meter (8.8 lb/A) of this inoculum results in 50% mortality of susceptible *C. sativa* the first year and increases in succeeding crops. The fungus can be seed-borne. Italian hemp varieties are resistant to the disease while all other varieties have been susceptible. The fungus causes disease only in *C. sativa*. No other kinds of plants have been infected. Because of the restricted host range, no special precautions are necessary for controlled use of the fungus.

Discussion

In absence of host plants *F. oxysporum* f. *cannabis* slowly declines in the soil. A relatively healthy crop of *C. sativa* might be grown in a

previously infested field after a number of years, possibly five or more, free of *C. sativa*. But the disease might build up again on succeeding crops. Where *C. sativa* is a weed in other crops, susceptible *C. sativa* plants would be eliminated. Although 'Iowa' (from escaped plants) was not as susceptible as 'Iran', it is not known if the surviving plants grown in inoculated soil were escapes or were genetically resistant. If they were genetically resistant, then the effectiveness of the fungus as a weed control agent would be diminished.

Since resistant varieties are known, it would always be possible to grow *C. sativa* in areas where the fungus had been introduced. The presence of resistant varieties might limit the length of time that the fungus would be useful in the control of illicit marijuana.

For biological control purposes, inoculum of the fungus could be introduced by air. It would not be necessary for complete coverage of a field since natural spread would occur by soil and plant debris movement and through the use of contaminated seed.

The biological control of *C. sativa* utilizing the fungus should be tested in fields where *C. sativa* is a weed and in fields where *C. sativa* is an illicit crop. The introduction of straw-soybean inoculum of the fungus by airplane would be considerably less expensive than overland travel and burning or otherwise disposing of the illicit crop. It would also have the advantage of not being necessary to return to the same area in succeeding years.

Downy mildew *(Pseudoperonospora cannabina)* was severe on 'Iran' variety in the Italian field trials. This fungus might also be used in a biological control program, and might even be used together with *F.*

oxysporum f. *cannabis*. The hemp downy mildew is not known to infect other plants but cross-inoculation studies with hemp and hop downy mildew (*P. humuli*) should be conducted since hop downy mildew has been reported from *C. sativa*. Production of inoculum of *P. cannabina* would be difficult since it would be necessary to grow the parasite on *C. sativa* plants and the sporangia are not long-lived. Plantings of infected *C. sativa* might be maintained upwind of areas where illicit *C. sativa* was grown.